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RESEARCH ARTICLE

Photoinduced Electron Transfer Reaction of 2-Thiobarbituric Acid and Methylene Blue: Mechanism and Kinetics

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Abstract: Barbiturates are known to have hypnotic properties and are used as active moiety on central nervous system. Depends up on the experiments conditions and functional group present on the drugs, make the drugs labile to photodecomposition. The photosensitized reaction of 2-thiobarbituric acid (2TBA) with methylene blue (MB) in alkaline medium using visible light has been studied. 2TBA exists in protonated and deprotonated form in equilibrium in alkaline medium. Photosensitized electron transfer from the deprotonated 2TBA to excited state MB takes place and the energy is transferred to 2TBA molecule which undergoes chemical reaction. The interconversion of protonated form to deprotonated form during the reaction is observed. The rate of the reaction has been calculated and the effect of pH, concentration of the sensitizer, the light intensity on the rate of the reaction has been studied. The quantum efficiency of the photo chemical reaction is calculated using potassium ferrioxalate actinometer and the effect of the concentration of the substrate on the quantum efficiency is calculated. The reaction mechanism and the excited states involved have been suggested.

Keywords: 2-Thiobarbituric Acid, Photosensitized reaction, Electron transfer reaction, Singlet - singlet energy transfer, Exciplex formation

Introduction

Photochemistry and photo stability of drugs presents the basic elements of science and serves as an excellent introduction to this emerging field of photochemistry. The photochemistry of drugs does not differ from that of the other molecules, since Pasteur[1] in 1846, scientists have been aware that many drugs are photo reactive, but until recently research in this area had been somewhat limited. However, until recently the matter has received only limited attention, also because it is generally feasible to protect the drug preparation from light through an appropriate package. As a result, the available knowledge is quit sparse. All pharmacopoeias mention that some drugs have to be protected from light, but this cautionary note is accompanied only by qualitative and incomplete information. However, since the introduction of acutely sensitive analytical methods, the realization of the need to identify the photochemical properties of a potential drug as early in its development as possible and the increased attention to the phototoxic effect of drugs, more details are becoming available.

It is generally easy to indicate in each drug molecule some feature that is likely to make it labile to photodecomposition but it is not easy to predict the rate, type and efficiency of the photochemical reaction of drug as it depends on the experiments conditions and functional group present on the drugs and this may lead to a significantly different photochemistry *in vivo* and *in vitro*. Fasani *et al*.[1] have discussed how the different functional groups (C=C, C=O, Aromatic compounds, heterocyclic compounds, products containing weak C-H bonds) behave in photochemical processes. Such functional groups are present in a very large fraction on of commonly used drugs. Thus, many drugs substances are expected to react when absorbing light. However, photodegradation of drug is of practical significance only when the compound absorbs significantly ambient light ($\lambda > 300$ nm). It is important to notice that most information about photoreaction available in the literature refers to the conditions where such processes are most easily observed and studied, *viz.* dilute solutions in organic solvents, whereas what matters for drug photo stability are (buffered) aqueous solutions or the solid state. Under such different conditions the photo reactivity of a drug may be dramatically different. As per one textbook example, benzophenone triplet - probably the most thoroughly investigated excited state - is a short lived species in organic solvents, (*e.g*. z ca 0.3 ps in ethanol) and is quite photo reactive via hydrogen abstraction under such conditions, and in general in an organic solution. However, the lifetime of this species increases by two orders of magnitude in water, where benzophenone is almost photo stable.

Photochemistry of 2-thiobarbituric acid

Thiobarbituric acid is a condensation product of malonic acid and thiourea which is closely related to barbituric acid. Barbiturates are known to have hypnotic properties and are used as active moiety on central nervous system. These compounds undergo two types of photochemical processes. The first one involves initial cleavage of the C-C bond and lead to an isocyanate[2]. This intermediate in turn adds nucleophilic solvents to give an amide in water and a urethane in ethanol. In variation of this process, a second C-C bond is cleaved and CO is eliminated. This leads to a hydantoin. The second general reaction is dealkylation to give products and this is the main path followed when one of the substituent is a stabilised alkyl group[3,4].

Thiobarbituric acid differs from barbituric acid only by the presence of a sulfur atom instead of an oxygen atom at the number 2 carbon. It is the parent compound of a class of drugs, the thiobarbiturates, which are analogous in their effects to barbiturates.

Biological activity of the 2TBA molecule is mainly related to the tautomerism and the nature of the substituent. 2TBA shows two pKa values one in the range 2.10 - 2.25 and a second in the range 10.55 - 10.72. The lower value corresponds to the molecular equilibrium between the *N*-protonated enol form and the neutral form, while the higher pKa is attributed to the equilibrium between neutral and anionic forms[5].

Thialbarbital (Intranarcon) is a barbiturate derivative synthesized in the 1960s. It has sedative effects, and was used primarily as the surgical anaesthesia. Thialbarbital is short acting and has less of a tendency to induce respiratory depression than other barbiturate derivatives such as pentobarbital. Thiobarbital is a drug which is a barbiturate derivative. It is the thiobarbiturate analogue of barbital. Thiobutabarbital (Inactin, Brevinarcon) is a short-acting barbiturate derivative synthesized in the 1950s. It has sedative, anticonvulsant and hypnotic effects and is still used as veterinary medicine for induction as surgical anaesthesia. Thiamylal (Surital) is a barbiturate derivative synthesized in the 1950s. It has sedative, anticonvulsant and hypnotic effects and is used as a strong but short acting sedative. Thamylal is still in current use, primarily for induction in surgical anaesthesia or as an

anticonvulsant to counteract side effects from other anaesthetics. It is the thiobarbiturate anologue of secobarbital. 2TBA is a substituted mercaptopyrimidine with three mobile H atoms and 10 possible tautomeric forms (Figure 1).

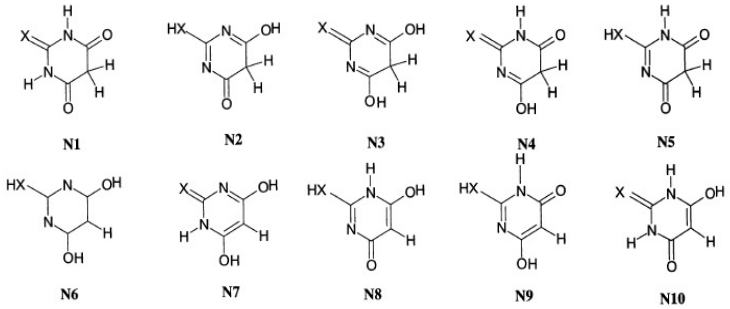


Figure 1. Isomers of 2TBA (X = S)

The present study reports the photochemical reaction of 2TBA using the cationic dye, methylene blue as photosensitizer in the aqueous alkaline medium on irradiation with visible light. The reaction is monitored by measuring the spectrum change of 2TBA. The kinetics of the photo degradation reaction has been studied. The effects of the pH, the concentration of the sensitizer, the concentration of the substrate and the light intensity have been evaluated on the rate of the reaction. The reaction has been used to determine the quantum efficiency and to know whether the reaction is monophotonic or biphotonic. The effect of the oxygen and free radical scavenger has been studied on the reaction. The mechanism of the photosensitized reaction has been suggested. Methylene blue has been used as a photosensitizer in the present study which has also been used as sensitizer in a number of photochemical reactions[6-9]. It shows two different type of energy transfer process *e.g*. electron transfer reactions, exciplex formations and energy transfer to triplet O_2 to convert it to singlet oxygen for photo-oxygenation.

Experimental

2TBA, methylene blue, potassium trioxalate ferrate, ammonium ferric sulphate, sodium acetate, phenanthroline were used as received. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were anayltical grade. Ethyl alcohol was HPLC grade. All chemicals were used as received. Water was doubly distilled. The solutions were kept in the dark at room temperature and were diluted as per the requirement.

100 W tungsten filament light source has been used for the exposure of the sample solution, convex lens is used to converge the irradiation and a glass water jacket is used to decrease the temperature of the solution. Light intensity is measured using "Light intensity measurement meter" (form Iwiss Solar, Model Number: TM206). All the spectral measurements have been done on UV-Vis spectrophotometer (Chemito - 2100). The pH were adjusted by addition of HCl or NaOH solutions, respectively and has been measured using pH-meter (Systronics, India) with glass calomal electrode.

The four sets of 2TBA solution (5.0×10^{-5} M) in alkaline aqueous medium were prepared. Two of them contain MB (1.5×10^{-5} M) as a photosensitizer. Two flasks, each containing methylene blue (MB) and without MB were kept in dark for 24 hours while remaining similar flasks were exposed to the visible light from 100 W tungsten lamp. The course of the reaction was followed by recording the spectrum of the exposed solution with a control solution in the range of 200-300 nm against reagent blank. The flask kept in the dark and the flask exposed without sensitizer did not show difference in the spectrum when compared to the control; while the exposed flask containing sensitizer showed shift in λ_{max}.

The effect of varying concentration of MB (in the range of 4×10^{-6} M to 3×10^{-5} M) and 2TBA (in the range of 3.5×10^{-5} M to 8.0×10^{-5} M) on the rate of the reaction has been studied respectively. The solutions were deaerated or saturated with O_2 by purging with nitrogen or O_2 respectively, for 30 min via a needle through the cap. The effect of the free radical scavenger on the photo sensitized reaction was studied by changing the medium from aqueous alkaline to methanolic alkaline. Light intensity can be varied by changing the distance of the sample solution from the tungsten lamp source, and intensity was measured using "Light intensity measurement meter". The study was carried out at room temperature nd pressure. The quantum efficiency of the photo reaction has been evaluated using potassium ferri oxalate actinometer.

Results and Discussion

Spectral characteristics

In agreement with literature[10], the UV spectrum of the 2TBA (5×10^{-5} M) recorded by us in aqueous solution exhibits two well-defined maxima one at 285 nm (ε = 24,000 L mol^{-1} cm^{-1}) and a second at 235 nm (ε = 9,000 L mol^{-1} cm^{-1}) at pH 2. The cationic and neutral species of 2TBA are in equilibrium between the pH value 2 to 6. On increasing the pH, an intense band at 235 nm [N4 or N5 or N10] (ε = 13,000 L mol^{-1} cm^{-1}) and a weak band at 285 nm [N1] (ε = 8,500 L mol^{-1} cm^{-1}) were observed at pH 12. 2TBA in alkaline solution shows higher absorbance at 235 nm and lower absorbance at 285 nm, while the protonation in the acidic solution shows higher molar absorptivity value at 285 nm and lower molar absorptivity value at 235 nm. The dominant peak in the acidic medium is at 285 nm which shifts to the shorter wavelength at 235 nm and becomes the dominant peak in alkaline medium at pH 12. The spectral changes are reversible by changing the pH of the solution.

Nam *et al.*[11] have reported that the concentration of 2TBA relative substance increases in the aged beef when the aged beef was treated with *e*-beam irradiation (2.5 kGy), but the difference between irradiated and non-irradiated long-term aged beef was not significantly different. However in the presence study 2TBA was found to be photo stable in acidic as well as in the alkaline medium, when irradiated by visible light. It was also found during the experiment that 2TBA and MB don't interact in the ground state but when the reaction mixture containing 2TBA (5×10^{-5} M) and MB (1.5×10^{-5} M) maintained at different pH between 2 to 12 were exposed to the visible radiation and the spectra were recorded against the reagent blank, the reaction mixture does not show any significant change in the pH range 2-6 but it undergoes photoreaction in the pH range of 8-12.

The changes of the absorption spectrum were plotted against time which show decrease at the λ_{max} 235 nm and 285 nm (Figure 2) and shows increase in the absorbance at 220 nm with time and an isobestic point was not observed. The product of photoreaction absorbs at a shorter wavelength in comparison to the starting material.

Determination of the rate constant and effect of different parameters on rate constant

The results of a typical run for the change in the absorbance of 2TBA with time have been presented in Figure 3A. The reaction follows the first order reaction kinetics as the plot of 2 + log OD (optical density) *vs*. time is a straight line with a positive slope (Figure 3B). The rate constant has been determined by the expression:

$$\text{Rate constant} = 2.303 \times \text{slope}$$

The half life of the reaction has been observed at different concentration of the substrate and $t_{1/2}$ value is constant over the above range of the substrate concentration. This suggests that the photochemical reaction is of the first order.

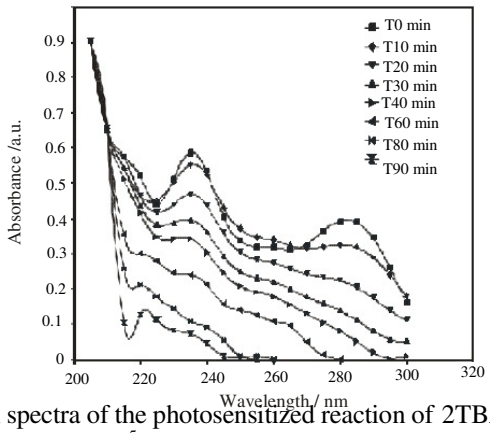


Figure 2. Absorption spectra of the photosensitized reaction of 2TBA with MB on exposure, 2TBA: 5 x 10^{-5} M, MB: 1.5 x 10^{-5} M, time interval: 10 min., 100 W tungsten lamp, pH 11

Effect of the pH

In this investigation, the effect of the pH value on the 2TBA photo degradation with MB in aqueous medium was studied in the pH interval range from 2 to 12. Figure 4 showed the dependence of k value for 2TBA degradation on the pH in the presence of 1.5×10^{-5} M MB, under 15×10^{8} E/s visible light intensity.

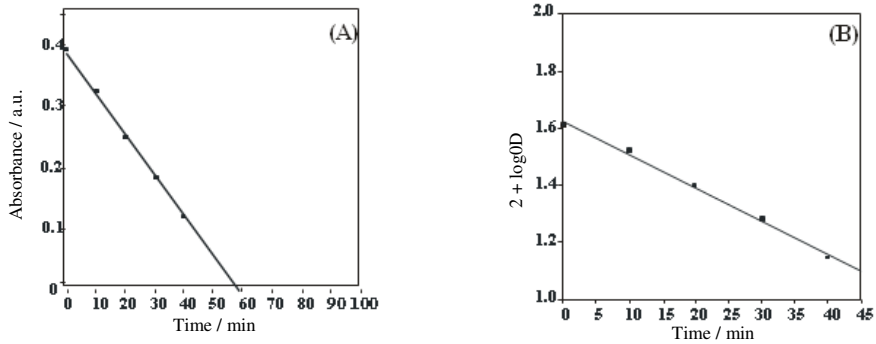


Figure 3(A). Absorption *vs.* time plot and (**B**) 2+log OD) *vs.* Time plot of the photosensitized reaction of 2TBA with MB at 285 nm on exposure, 2TBA: 5x10 $^{-5}$ M, MB: 1.5x10^{-6} M, Time interval: 10 min., Source: 100 W tungsten lamp

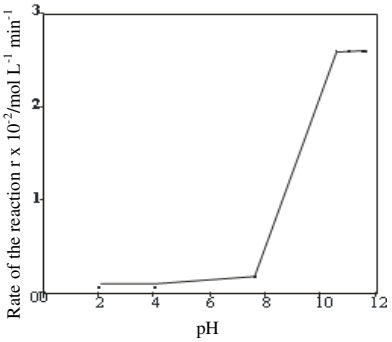


Figure 4. Effect of the pH on the rate of the reaction measured at 285 nm, 2-TBA: 5x10^{-5} M, MB: 1.5x10^{-5} M, Time interval: 10 mins, Source: 100 W tungsten lamp

The spectrum of 2TBA was recorded at several pH values ranging from 2 to 12, while keeping the concentration of 2TBA as constant. The absorbance at λ_{max} 285 nm was plotted against the pH of the solution. At different pH and pKa value, the total concentration of 2TBA was shared among the cationic, neutral and anionic species. The neutral species of HTBA can be protonated or de-protonated depending on the pH value of the solution.

$$HTBA + H^+ \leftrightarrow H_2TBA^+ (pH\ 2 - 6)\ \log K1 = -10.16$$

$$HTBA \leftrightarrow H^+ + TBA^- (pH\ 8 - 10)\ \log K2 = -1.874$$

2TBA exists as H_2TBA^+ and HTBA forms between pH value 2 - 6 which show λ_{max} at 235 nm (ε = 9,000 L mol^{-1} cm^{-1}) and at 285 nm (ε = 24,000 L mol^{-1} cm^{-1}) respectively. 2TBA exists as HTBA and TBA^- forms between pH 8 - 12 which show λ_{max} at 235 nm (ε = 13,000 L mol^{-1} cm^{-1}) and at 285 nm (ε = 8,500 L mol^{-1} cm^{-1}) respectively. The rate of the photosensitized reaction increases with increase in pH and becomes constant at pH 10 suggests that only TBA^- species of 2TBA undergo photo reaction.

The spectrum (Figure 2) shows decrease in the absorbance at the λ_{max} of both HTBA and TBA^- species which remain in equilibrium. The absorbance at the λ_{max} 285 nm (k = (2.59 $\pm$ 0.06) × 10^{-2} min^{-1} at 285 nm) decreases faster than absorbance at λ_{max} 235 nm (k = (1.23 $\pm$ 0.04) × 10^{-2} min^{-1} at 235 nm). The absorbance decreases at 285 nm is due to the photo degradation reaction of 2TBA anionic form to form SCN^- as photo product, while the absorbance decrease at 235 nm is due to the conversion of HTBA to TBA^- form to maintain the equilibrium. As the reaction proceeds the concentration of the TBA^- species decreases and the equilibrium of HTBA $\leftrightarrow$ H^+ + TBA^- is shifted to right side and the HTBA species converts to the TBAspecies to maintain the equilibrium.

Effect of the initial concentration of sensitizer

Figure 5 showed the dependence of 2TBA photo degradation on the initial concentration of sensitizer under 15 × 10^8 E/s visible light irradiation. Figure showed the dependence of the first kinetic constant (k) on the sensitizer concentration. The results showed that the photo degradation of 2TBA did not occur in the absence of MB. However, 2TBA degradation could be efficiently enhanced in the presence of MB. 2TBA has it's λ_{max} below 400 nm and is not photodegraded directly by the visible light. Methylene blue absorbs visible radiation at 665 nm and gets excited, which on collision with the 2TBA, transfers energy to it. The first-order kinetic constant (k) for 2TBA degradation increased with increasing sensitizer concentration then becomes constant with increasing sensitizer concentration. However, the first-order kinetic constant for 2TBA degradation decreased as the MB concentration reached above a threshold level where the self deactivation of the sensitizer molecule takes place by intermolecular collision[12]. Therefore, the optimal sensitizer concentration value was 1.5×10^{-5} M which mostly favored the photo degradation of 2TBA in the experimental condition.

Effect of the initial concentration of substrate

The first-order kinetic constant k remains the same (5.76 ± 0.04) × 10^{-2} min^{-1} with increasing substrate concentration from 3.5×10^{-5} M to 8×10^{-5} M and decreases slightly at the higher concentration of substrate. Obviously, there should be an optimal concentration of substrate (5×10^{-5} M) for 2TBA degradation. The first-order kinetic constants k remaining constant in the concentration range shows that the reaction is independent of the initial concentration of the substrate[13].

Effect of the anaerobic condition

Otsuji *et al.*[14] reported photochemical hydrolysis of barbital (5,5-diethylbarbituric acid) in

aqueous solution. The reaction was studied in the anaerobic condition to observe the participation of singlet oxygen during the photosensitized reaction.

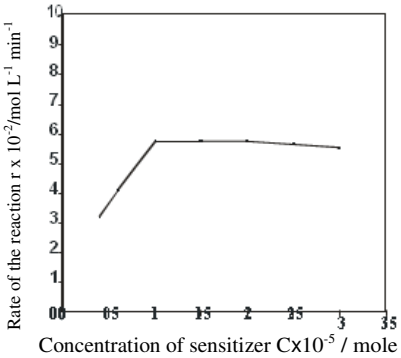


Figure 5. Effect of the sensitizer concentration on the rate of the reaction at 285 nm, 2TBA: $5x10^{-5}$ M, MB: $4x10^{-6}$ M to $3x10^{-5}$ M, Time interval: 10 min, Source: 100 W tungsten lamp, pH 11

The cationic dye methylene blue - photo sensitizer - absorbs the visible radiation and goes to the singlet excited state and is converted to the triplet state by ISC (Intersystem Crossing). The excitation energy of the sensitizer molecule is transferred to $^3O_2$ molecule and converts it to the singlet state, while photo sensitizer molecule returns to the ground state. This singlet oxygen is a highly reactive species which gives the photo oxygenation reaction. Methylene blue is reportedly efficient sensitizers for the conversion of triplet oxygen to singlet oxygen in the presence of light[15-19]. The first-order kinetic constant was calculated for the exposed solutions of the different concentration of the substrate [2TBA] and the sensitizer MB in the aerobic as well as in the anaerobic condition. The first-order kinetic constant in the anaerobic condition $(1.23 \pm 0.04) \times 10^{-2}$ min^{-1} and $(2.59 \pm 0.06) \times 10^{-2}$ min^{-1} at 235 nm and 285 nm respectively, does not show change and remains the same [$(1.23 \pm 0.05) \times 10^{-2}$ min^{-1} and $(2.59 \pm 0.05) \times 10^{-2}$ min^{-1} at 235 nm and 285 nm respectively) as in the aerobic condition. The φ value of the reaction was also calculated in the anaerobic condition of the reaction which remains constant and same as in the aerobic condition. However the experimental result suggests that the singlet oxygen does not participate in the photosensitized reaction of 2TBA.

Effect of the free radical scavenger

The formation of free radical during the photosensitized reaction of 2TBA was studied by changing the medium from aqueous alkaline to methanolic alkaline. The solutions of the different concentration of substrate [2TBA] and the sensitizer MB were prepared in the alkaline methanolic solution and irradiated with the visible light. The two isomers of 2TBA, N1 and N10 show λ_{max} at 235 nm and at 285 nm are present approximately in the same concentration in the alkaline methanolic solution. The first-order kinetic constant for the 2TBA degradation reaction in the aqueous alkaline medium was $(1.23 \pm 0.04) \times 10^{-2}$ min^{-1} and $(2.59 \pm 0.06) \times 10^{-2}$ min^{-1} at 235 nm and 285 nm respectively doesn't show change in the alkaline methanolic solvent except the rate of the reaction slightly decreases to $(1.02 \pm 0.03) \times 10^{-2}$ min^{-1} and $(2.23 \pm 0.03) \times 10^{-2}$ min^{-1} at 235 nm and 285 nm respectively in the alkaline methanolic solvent. The photochemical reaction does not show significant decrease in the alkaline methanolic solvent suggests that there is no free radical formation during the reaction[20].

Effect of the light intensity

Light intensity is another important factor to be considered during photochemical process.

Generally, higher light intensity can lead to higher degradation rate for organic pollutants in photochemical reaction. The same results were obtained in this investigation as shown in Table 1. The solutions of the different concentration of the substrate [2TBA] and the sensitizer MB were prepared in the aqueous alkaline solution and irradiated with the visible light of the different intensity. The increase of the light intensity [Einstein / second (E/s)] shows positive effect and the first-order rate constant of the reaction increases as the light intensity increases. The increase in the number of the photons increases the number of the excited sensitizer molecule and the rate of the reaction also increases. A linear relationship has been observed between the light intensity and the first-order rate constant of the reaction.

Table 1. Effect of light intensity on quantum efficiency and on the rate of the reaction at 285 nm, 2TBA: $5x10^{-5}$, MB: $1.5x10^{-5}$ M, Time interval: 10 min, Source: 100 W tungsten lamp, 11 pH

Light intensity ($Ix10^{8}$) E/s	Rate of the reaction at 285 nm ($rx10^{-2}$) mole / L min [r ± 0.5]	Quantum efficiency
5	0.94	0.3236
10	1.84	0.3223
15	2.62	0.3216
20	3.46	0.3253
25	4.55	0.3263

The quantum efficiency

The quantum efficiency of the photoreaction of 2TBA and MB has been determined using Potassium ferrioxalate actinometer under various initial concentrations of 2TBA. The plot of the φ value and the initial concentration of the 2TBA (Figure 6A) shows a horizontal relationship. The kinetic expression for the inverse of quantum yield based on steady state approximation and given by the equation:

Equitation shows that the quantum yield of the reaction is independent of the substrate concentration. The plot of 1/φ *vs.* 1/ C (Figure 6B) gives a horizontal graph with zero slope indicating a singlet - singlet energy transfer mechanism between excited state MB molecule to 2TBA anion and also suggests that there is no exciplex formation takes place during the photosensitized reaction[21]. The singlet excited state of the anionic 2TBA molecule undergoes decomposition to give photo product[22]. The φ value of the reaction is low (Φ = 0.32); the process of the deactivation of the excited state is predominant as compared to the decomposition of the excited state of the substrate molecule. The photo reaction product formation is monophotonic process as the quantum efficiency does not change with increase in light intensity.

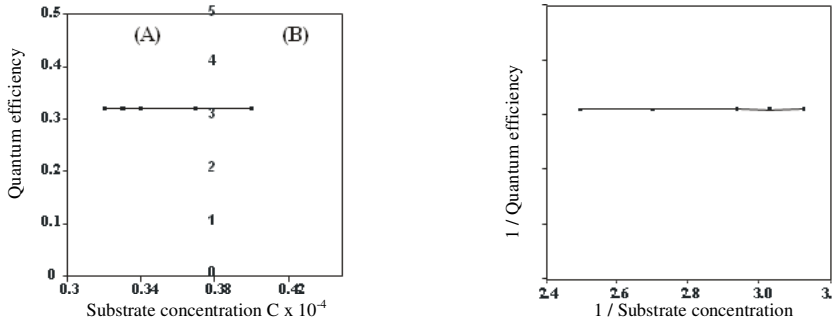


Figure 6. Plot of (A) quantum efficiency *vs.* substrate concentration (B) inverse of the quantum efficiency *vs.* inverse of the substrate concentration at 285 nm, 2TBA: $3.3x10^{-5}$ M to $3.9x10^{-5}$ M, MB: $1.5x10^{-5}$ M, Time interval: 10 min, Source: 100 W tungsten lamp, 11 pH

Identification of photolysis products

The reaction mixture after exposure to the visible radiation for 90 mins was analyzed for the reaction product. The photoproduct shows only one absorbance maxima in UV spectrum at 220 nm having the molar absorptivity of 2540 L mol^{-1} cm^{-1}.

The experimental study shows that photo reaction does not involve singlet oxygen. The substrate molecule does not undergo oxidation. The effect of the free radical scavenger shows that there is absence of the free radical formation in the reaction. The singlet excited state ofthe substrate molecule directly undergoes degradation forming the product. The analysis of the reaction mixture for photo product does not show the presence of organic compound. The qualitative test for SO_{4-2} and S^{-2} was found negative with $BaCl_2$ solution and lead acetate respectively. The qualitative analysis, spectrophotometric analysis and IC (Ion Chromatography) analysis suggest the presence of SCN^- which appears to be the only photo product.

Test for the SCN^- ion

The reaction mixture was further tested for SCN^- ion with $FeCl_3$ solution which gave an orange colouration which absorbed at 440 ± 2 nm. This was further confirmed by preparing a standard solution of KCNS and MB of the same concentration as in the experiment with 2TBA and MB at 11 pH. The spectrum of the solution of SCN^- with MB was measured before exposure and after the exposure to light. (Figure 7).

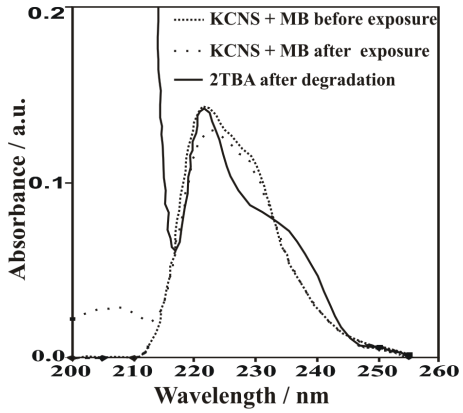


Figure 7. Absorbance spectra of KCNS with MB before and after exposure and the reaction product, KCNS: $5x10^{-5}$ M and 2TBA: $5x10^{-5}$ M, MB: $1.5x10^{-5}$ M, Source: 100 W tungsten lamp

The absorbance spectra of KCNS with MB of the same concentration range as of 2TBA before and after irradiation shows the absorbance maximum at 225 nm and 220 nm having the molar absorptivity of 2,472 L mol^{-1} cm^{-1} and 2,674 L mol^{-1} cm^{-1} respectively (Table 2).

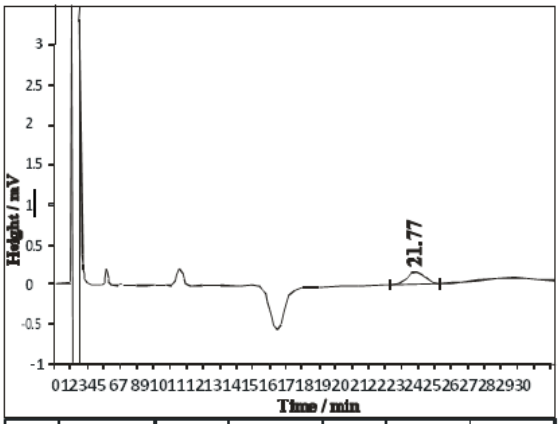
Table 2. λ_{max} and molar absorptivity of photoproduct and [KCNS + MB] before and after exposure at 11 pH KCNS: $5x10^{-5}$ M and MB: $2x10^{-5}$ M

Species Present	λ_{max}	Molar absorptivity L mol^{-1} cm^{-1}
[KCNS + MB] Before exposure	225 nm	2472
[KCNS + MB] After exposure	220 nm	2674
Reaction product	220 nm	2540

IC analysis for thiocyanate determination

Thiocyanate was extracted from the reaction mixture by shaking for 10 min with 5 g of charcoal. A minimum of two replicates were extracted for all analyses. The resulting solution extract was filtered through Whatman no 42 filter paper and refrigerated until IC analysis. Figure 8 shows the IC analysis spectrum of reaction photo product of 2TBA. The RT (retention time) of the reaction product at 21.77 min matches with the RT of the standard solution of SCN^-. The excited state molecule of 2TBA undergoes decomposition to give SCN^- as photo product.

Miura *et al*.[23] have reported that the sulfur anions have high absorbance in the range of 200-230 nm and they have reported the absorbance spectra of thiocyanate anion. Chiang and Kresge[24] have reported the change in the absorbance of the thiocyanate ion at 220 nm, measured in the concentrated aqueous perchloric acid solution. Jochym *et al*.[25] have reported the UV irradiation of the barbital yields derivative of urea and biuret and interpreted that the origin of the IR band at 2170 cm^{-1} is of the isocyanate intermediate which is formed after the pyrimidine ring opening.



No	Retention Sec	Height mV	Area mV * Sec	Area	Conc mg/L	Amount
1	21.77	0.13	9.411	100	0	0

Figure 8. IC analysis graph of photo product of 2TBA

Mechanism

The 2TBA exists as neutral and anionic species in equilibrium in the alkaline medium and have λ_{max} at 235 nm and 285 nm which do not absorb visible light and are photo stable. Methylene blue (MB) has λ_{max} at 665 nm which absorbs visible radiation and forms singlet excited molecule. Some of the singlet excited MB can deactivate to ground state by radiation deactivation. Singlet excited cationic MB molecule acts as electron acceptor and singlet - singlet energy transfer excites the 2TBA anion to singlet excited state by electron transfer mechanism which undergoes decomposition. Deactivation of some of the singlet excited TBAmolecule to ground state occurs because the φ value of the reaction is low ($\Phi = 0.32$).

The excited TBA^- molecule undergoes decomposition by reorganisation to give SCN^- as a photo product. The photo product SCN^- is an anion which appears to undergo an electron transfer reaction with the excited molecule of MB. The electron transfer from SCN^- to MB result in the formation of an $[SCN^-MB^+]$ exciplex which shows increased

absorbance and hypsochromic shift in comparison to the solution of the same concentration of SCN^- and MB in the ground state (Figure 7).

As the reaction proceeds the concentration of the anionic species of the 2TBA species decreases and the equilibrium of shifts to right side and the HTBA species converts to the TBA^- species to maintain the equilibrium (Scheme 1).

$[MB^+] + h\nu \longrightarrow$	$[MB+]^{*1}$	Irradiation	(1)
$[MB^+]^{*1} \longrightarrow$	$[MB^+] +$ heat	Deactivation	(2)
$[MB^+]^{*1} + [TBA^-] \longrightarrow$	$[MB^+] + [TBA^-]^{*1}$	Energy Transfer by Electron Transfer	(3)
$[TBA^-]^{*1} \longrightarrow$	$[TBA^-]$	Deactivation	(4)
$[TBA^-]^{*1} + [TBA^-] \longrightarrow$	2TBA	Concentration Quenching	(5)
$[TBA^-]^{*1} \longrightarrow$	$[SCN^-]$	Decomposition by Reorganization	(6)
$[SCN^-] + [MB^+]^{*1} \longrightarrow$	$[SCN^-MB^+]$	Electron Transfer Exciplex Formation	(7)

Scheme 1. Mechanism of 2TBA degradation

Conclusion

2TBA exists as neutral and anionic species in equilibrium in the alkaline medium. The irradiation of MB excites it to singlet excited state which transfers it's energy to 2TBA anion and returns back to the ground state. The excited TBA^- molecule undergoes decomposition by eorganization to give SCN^- as a photo product. Thiocyanate[26] is known to be an important part in the biosynthesis of hypothiocyanite by a lactoperoxidase[27-29]. Thus the complete absence of thiocyanate in the human body is damaging to the human host defense system. But its continued ingestion can cause goiter.

The first-order kinetic constant (k) of the photodecomposition reaction of 2TBA is independent of the concentration of 2TBA and MB but it is dependent on the pH of the solution and the intensity of light. The plot of $1/\varphi$ *vs.* 1/C suggests that singlet – singlet energy transfer occurs from the sensitizer to the substrate without exciplex formation.

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